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Comparative study to evaluate vaginal fluid creatinine and AFI for diagnosis of premature rupture of membranes

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Abstract
Aim: To evaluate vaginal fluid creatinine and AFI for diagnosis of Premature rupture of membranes.
Material and Methods: A total of 80 pregnant women were enrolled in the study Group 1 (control group) consisted of 40 healthy women with no history of leaking per vaginum or leak detected per speculum or per vaginum examination and Group 2 (case group) consisted of 40 women with history of leaking per vaginum or leak detected per speculum or per vaginum examination. All patients subjected to ultrasonography for Amniotic Fluid Index (AFI) measurement. All patients were sampled for vaginal fluid creatinine by speculum examination. Sample processed. AFI & Vaginal fluid creatinine level was estimated & compared for its significance. Receiver operating characteristic (ROC) curve analysis was used to establish the optimal cut-off concentrations for vaginal fluid creatinine & AFI, its accuracy, sensitivity & specificity.
Result: The mean vaginal fluid creatinine in case group was 0.42±0.1 mg/dl and 0.18±0.08 mg/dl in control group respectively and its difference was statistically significant ($p<0.001$).The optimal cut off value was >0.25 mg/dl with 90% sensitivity, 87.5% specificity & 91.6% accuracy for diagnosis of PROM. The mean value of amniotic fluid index (AFI) was significantly lower in the case group than in the control group (5.3±1.7 vs. 11.3±1.5, $p < 0.001$). AFI was found to have highly significant association between the groups. (<0.001). The sensitivity & the specificity of amniotic fluid index (AFI) to diagnose PROM (case) were 97.5% & 92.5% respectively, while it's over all accuracy was 99.3% respectively, with a cut-off value of ≤ 7.5 cm.
Conclusion: So to conclude that if on clinical examination diagnosis of PROM is doubtful or suspicious, USG for AFI can be first screening investigation that can be done to confirm leaking per vaginum, however there are other causes of reduced AFI or oligohydramnios such as placental insufficiency etc, so by adding vaginal fluid creatinine which is one of the diagnostic test for PROM we can increase the sensitivity and specificity to diagnose PROM. In patients with insignificant leaking and decreased AFI and with suspicion of PROM, vaginal fluid creatinine estimation may be useful for definitive diagnosis & institute appropriate management. Estimating vaginal fluid creatinine levels and AFI may be useful to make an accurate diagnosis of PROM even at rural health care facilities and ensure early referral to tertiary care centres if needed.

Keywords: Creatinine, premature rupture of membranes, Prelabor rupture of membranes

Introduction
Premature rupture of membranes (PROMs) constitutes one of the most important dilemmas which are difficult to diagnose in obstetric practice. Premature rupture of membranes is defined as spontaneous rupture of fetal membranes beyond 28 weeks of pregnancy but before the onset of uterine contractions. PROM is now known as Prelabour rupture of membranes. If PROM occurs before the 37 completed gestational week, it is called preterm PROM (PPROM) and accounts for about one fourth of all cases of ruptured membranes [1]. PROM occur in 8.0–10.0% of all pregnant women at term. A prolonged interval leads to increased maternal & fetal complications.60.0-80.0% of PROM happens in term pregnancies and 20.0-40.0% in pregnant women before the 37th week [1].
Despite the advances in medicine and technology, PROM and especially PPROM are clinical condition associated with adverse prognosis of both the mother & fetus. Increased perinatal morbidity & mortality causes include prematurity, perinatal infections, umbilical cord compression, oligohydramnios, pulmonary immaturity. In addition, there are maternal risks such as increased caesarean section rate, choriodecidual infection, placental Abruption,

retained placenta, endometritis, maternal sepsis & even death. Numerous risk factors are associated with PROM such as Smoking, history of sexually transmitted infections, lower socioeconomic status, vaginal bleeding, previous preterm delivery, polyhydramnios, multifetal pregnancy and after procedures like cerclage, and amniocentesis [3]. Current data suggest that in 47.0% of cases, clinicians are unsure about the diagnosis of PROM on the basis of patient history alone and clinical examination by sterile speculum examination [4]. A misdiagnosis often leads to a series of unnecessary or inappropriate interventions that may be harmful to both mother and fetus.

The conventional minimally invasive criteria for diagnosis of PROM is based on clinician's ability to see for three clinical signs on per sterile speculum examination:

1. Pooling of clear fluid seen in the posterior fornix of the vagina or leakage of the fluid seen coming through cervical os on per speculum examination.
2. Whether the discharge changes nitrazine paper from yellow to blue that indicate alkaline pH of cervico-vaginal discharge; and/or
3. Microscopic ferning seen on slide prepared from cervico vaginal discharge [5, 6].

Although diagnosis of PROM can be easily made in the presence of obvious rupture of membranes while the conventional diagnostic interventions in suspected cases of PROM result in many false positive and false negative results that lead to unnecessary interventions such as hospital admission and induction of labor [7].

Many biochemical diagnostic markers for PROM have been described, like measurement of vaginal PH, insulin growth factor binding protein-1(IGFBP-1), fetal fibronectin tests, alpha fetoprotein (AFP), human chorionic gonadotropin (HCG), prolactin, creatinine, urea [5]. Amnisure test which detect PAMG-1 in cervicovaginal fluid has the best sensitivity and specificity. Inspite of improved diagnostic potential of these markers, they have not become popular due to their high cost and complexity [5].

The fetus starts to excrete urine in amniotic fluid at 8-10th weeks of gestation and fetal urine is major component in amniotic fluid in second half of pregnancy and therefore creatinine measurement can be done for diagnosis of premature rupture of membranes [8].

AFI was estimated by a four quadrant technique, which was sum of deepest, unobstructed, and vertical length of pocket of fluid in each quadrant. Amniotic fluid index (AFI), a widely used method for evaluation of fetal well–being, is considered a useful parameter in this regard, given its potential in predicting adverse outcomes, helping to decide on optimal mode of delivery in pregnancies complicated by PPROM or PROM. Accordingly, presence of oligohydramnios (AFI < 5 cm) after PPROM has been suggested to be associated with increased likelihood of adverse fetal (*i.e.* intrauterine growth restriction, fetal distress, pulmonary hypoplasia, respiratory distress syndrome, neonatal sepsis, necrotizing enterocolitis, intraventricular hemorrhage, and bronchopulmonary dysplasia) and maternal (*i.e.* chorioamnionitis) perinatal outcomes, contributing to an increase in neonatal sepsis and mortality. Ultrasound has emerged as a useful tool in detecting and predicting prognostic factors. Amniotic fluid index –especially low AFI has been used as a predictor for adverse outcome by many obstetricians and several studies support its use as a tool to decide on optimal mode of delivery. Also in making decisions regarding referral to a higher centre with better neonatal facilities [9]. Sonography may be used to confirm PROM and may contribute in the diagnosis of PROM but is not 100% sensitive & specific. Sonographic oligohydramnios may not be present in PROM, if ROM is of less duration or recent onset.

The management of PROM patients remains controversial. Therefore, an accurate and early diagnosis of PROM becomes important to formulate management plan in these patients [1, 8].

The goal of this study was to evaluate vaginal fluid creatinine and AFI for diagnosis of premature rupture of membranes.

Aim: To evaluate vaginal fluid creatinine and AFI for diagnosis of premature rupture of membranes & to evaluate its reliability by comparison of creatinine level in vaginal fluid &AFI in both groups and to determine the cut off values of and its clinical utility.

Material and Methods

This is a prospective case control study conducted on all patient presenting to the department of Obstetrics & Gynaecology, Shri ram murti Smarak institute of medical sciences, Bareilly in the labour room were recruited over a period of one and half years from November 2019 to April 2021.

Inclusion criteria

1. Antenatal women with single pregnancy
2. Gestational age between 37 to 42 weeks without fetal congenital anomaly
3. Without any severe medical illness

Exclusion criteria

1. PROM in patient having multiple pregnancy
2. Congenital fetal anomaly
3. Vaginal bleeding or spotting
4. History of vaginal infection
5. Meconium stained liquor
6. Intrauterine fetal demise
7. Women not willing to participate in the study

A total of 80 pregnant women who met above criteria were enrolled. Group 1(Control group) consist of 40 healthy term pregnant women with no history of leaking per vaginum or leak detected per speculum or per vaginal examination and Group 2(study group) consist of 40 pregnant women with clinical diagnosis of PROM with history of leaking per vaginum or confirmed leak detected on per speculum or per vaginal examination.

The Vaginal wash samples collected from subjects placed in the lithotomy position while maintaining good illumination. In the control group 5ml of sterile NS injected into the posterior fornix and 3ml of vaginal wash fluid aspirated using the same syringe. In the study group 5ml of sterile NS injected into the posterior fornix where leak was minimal and atleast 3 ml of vaginal aspirate collected using the same syringe and directly fluid aspirated in which frank leak was present using syringe. Creatinine level was measured by Modified Jaffe chemical calorimetric method using the Mindray BS 480 analyser. Amniotic fluid index of all patients were calculated and analysed in both groups.

Observations

Demographic Profile and clinical characteristics

Table 1: Comparison of Demographic Profile in both groups

Parameters Mean + SD/Percentage#	Groups		P value*
	Case (N=40)	Control (N=40)	
Age (years)	24.98±3.5	25.70±4.6	0.433
Gravida	1.68±0.9(60%)	2.03 ±1.1(52.5%)	0.446
Parity	0.5±0.7	0.7±0.8	0.499
Occupation	Housewife (92.5%)	Housewife (90 %)	0.260
Socioeconomic status	Lower class (80 %)	Lower class (72.5 %)	0.431
Booking status	Booked (50%)	Booked (45%)	0.654
BMI(Kg/m^2)	23.3 ± 2.2	22.7 ±3.5	0.361
Gestational Age(weeks)	38.43 ±1.2	39.0 ±1.4	0.042

*Chi square test,# Independent sample t test

Majority of patients in both groups were in the age group of 21-25 years. Majority of patients in both groups were primigravida, housewives belonging to low socioeconomic status. Majority of mothers were in gestational age between 37-38+6 weeks in case group and 39-40+6 weeks in control group. The association was found to be non-significant in above parameters in both groups (P >0.05)

Table 2: Comparison of clinical parameters in both groups

Parameters (Mean + SD)#	Groups		P value*
	Case (N=40)	Control (N=40)	
Pulse rate (BPM)	87.5±13.5	88.2±7.6	0.775
SBP (mmHg)	123.4±8.7	122.1±9.9	0.534
DBP (mmHg)	80.1±5.0	78.3±6.4	0.165
Temperature (Fahrenheit)	98.6±1.1	97.7±0.6	<0.001

*Chi square test,# Independent sample t tes

Comparative analysis of clinical parameters in both case & control groups did not show any statistical significance for pulse rate, respiratory rate and blood pressure. The mean temperature observed in case group was 98.6 F and 97.7 F in control group and this was calculated to have a p value of <0.001 which is highly significant.

Table 3: Comparison of blood parameters in both groups

Parameters (Mean + SD)#	Group		P value*
	Case (N=40)	Control (N=40)	
Hemoglobin (gm/dL)	11.2±1.4	10.8±1.5	0.221
TLC (cumm)	12014.0±2782.1	9399.90±2148.2	<0.001
Platelet count (×1000/mm^3)	216.98±26.70	199.03±27.87	0.004

*Chi square test,# Independent sample t test

Blood parameters like haemoglobin, TLC, DLC, platelet counts were studied in which TLC (cumm) count among the cases was found statistically significant in comparision to control group with average TLC count of 12,014 cumm in cases as against 9,399.90 cumm in controls with p value of <0.001.The mean difference in platelet count in both the groups was statistically significant with average platelet count of 216.98(x1000/ mm^3) in cases and 199.03 (x1000/ mm^3) in controls with p value of 0.004.Further investigation and studies are required considering the importance or significance of platelet, TLC, platelet/leucocyte ratio(PLR) as marker for prediction or diagnosis of PROM.

Table 4: Comparison and distribution of creatinine level in vaginal fluid in both groups

Vaginal fluid creatinine (mg/dl)	Group		P value*	P value*
	Case (N=40)	Control (N=40)		
<= 0.1	3 (7.5%)	15 (37.5%)	0.002	<0.001
0.11 - 0.2	1 (2.5%)	20 (50.0%)	<0.001	
0.21-0.3	7 (17.5%)	4 (10.0%)	0.329	
0.31-0.4	11 (27.5%)	1 (2.5%)	0.002	
>0.4	18 (45.0%)	0 (0.0%)	<0.001	
Mean±SD#	0.42±0.1	0.18±0.08	<0.001#	<0.001

*Chi square test, # Independent sample t test

In our study, the mean value of vaginal fluid creatinine was 0.42 ±0.1mg/dl in case group and 0.18±0.08 mg/dl in control group respectively. The vaginal fluid creatinine in 90.0% of patients in case group was 0.21- 0.4 mg/dl range and in 87.5% of patients in the control group, the range was 0.06-0.2 mg/dl. The difference in vaginal fluid creatinine was higher in study group as compared to control group and it was highly significant with p value of <0.001. On analysis of ROC curve, the sensitivity, specificity and accuracy of vaginal fluid creatinine for diagnosis of PROM was 90%,87.5% and 91.6 % respectively with cut off value of > 0.25 mg/dl.

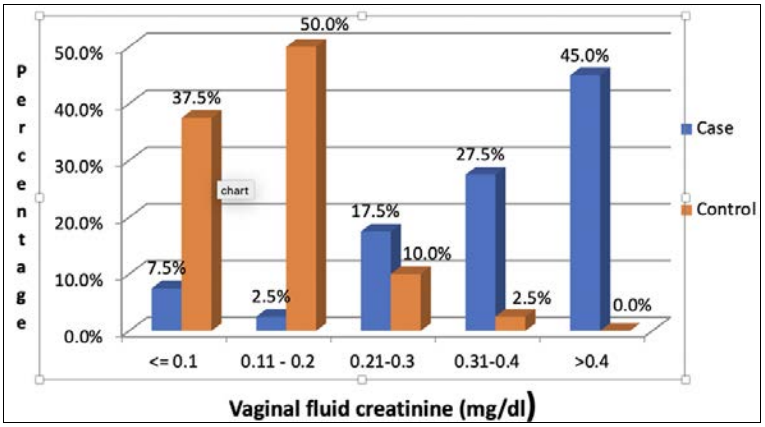

Fig 1: Comparison and distribution of creatinine level in vaginal fluid in both groups

Table 5: Comparison and distribution of amniotic fluid index (AFI) in both groups

AFI (in cms)	Group		P value*	P value*
	Case (N=40)	Control (N=40)		
<5	13 (32.5%)	0 (0.0%)	<0.001	<0.001
5-9	27 (67.5%)	4 (10.0%)	<0.001	
10-14	0 (0.0%)	36 (90.0%)	<0.001	
≥15	0 (0.0%)	0 (0.0%)	--	
AFI (cm)#	5.3±1.7	11.3±1.5	<0.001#	<0.001#

#= Mean±SD, *Chi square test

The mean value of amniotic fluid index (AFI) was significantly lower in the case group than in the control group (5.3±1.7 vs. 11.3±1.5, p < 0.001). AFI in 5-9 cms range, was noted in a majority of 31 (67.5%) patients in case group and only 4 (10.0%) in controls. 10-14 cms amniotic fluid index was found in 0 (0.0%) cases and a great majority of 36 (90.0%) in controls. AFI was found to have highly significant association between the groups. (<0.001).

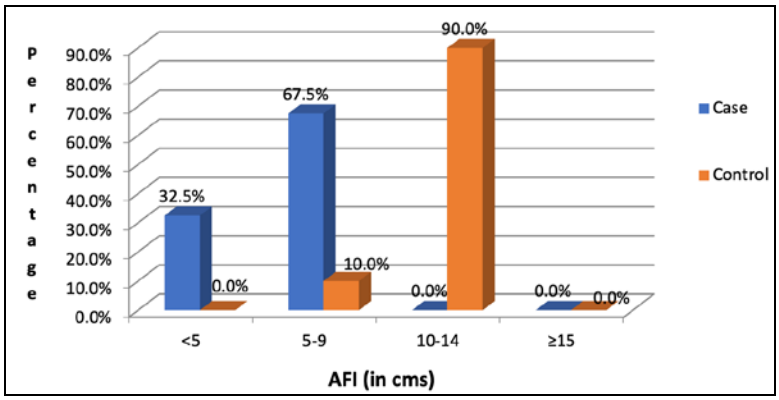

Fig 2: Comparison and distribution of amniotic fluid index (AFI) in both groups

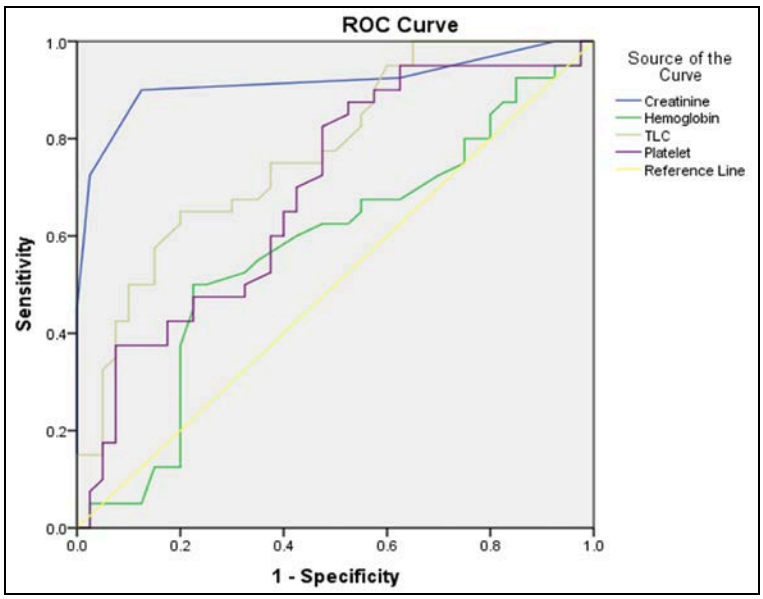

Fig 3: ROC curve of Blood parameters and vaginal fluid creatinine

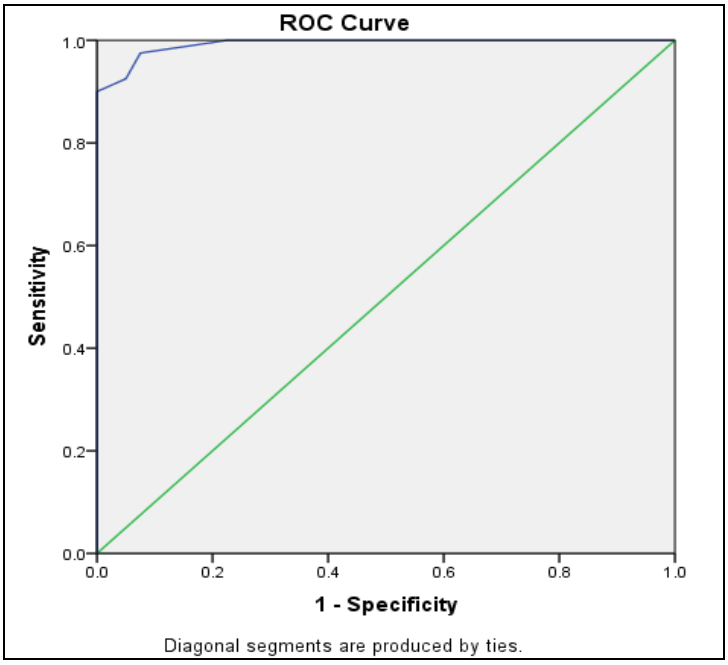

Fig 4: ROC curve of AFI

Receiver operating characteristic (ROC) curve analysis was used to establish the optimal cut-off concentrations for vaginal fluid creatinine, hemoglobin, TLC and AFI. The sensitivity & the specificity of vaginal fluid creatinine to diagnose PROM (case) were 90% & 87.5% respectively. While it's overall accuracy was 91.6%, with a cut-off value > 0.25 mg/dl. The sensitivity & the specificity of amniotic fluid index (AFI) to diagnose PROM (case) were 97.5% & 92.5% respectively, while it's over all accuracy was 99.3% respectively, with a cut-off value of ≤ 7.5 cm. TLC accuracy was 77.4%, with a cut-off value >9230/cumm, with sensitivity and specificity of 77.5% and 52.5% respectively.

Table 6: Correlation of AFI and vaginal fluid creatinine in case group

AFI (cm)	Total no. of patients	Mean AFI (cm)	Vaginal fluid creatinine(mg/dl)			P value
			0.1-0.2	0.3-0.4	0.5-0.6	
1-5	19	4.15		11(27.5 %)	8 (20%)	<0.001
5-10	21	6.23	3(7%)	7 (17.5%)	11(27.5%)	<0.001

In our study 27.5 % patients with sonography suggestive of AFI of 1-5 cm had vaginal fluid creatinine in the range of 0.3-0.4 mg/dl which was found to be highly significant. Another In our study 27.5 % patients with sonography suggestive of AFI of 5-10 cm had vaginal fluid creatinine in the range of 0.5-0.6 mg/dl which was found to be highly significant.

Discussion

In our study, the mean maternal age of case group (with PROM) was 24.98±3.5 years and 25.70±4.6 years in control group (without PROM). The difference of mean was not significant between groups (P>0.05) with a range of 19-38 years. Similarly, Kariman N *et al.* in their study also found the mean ages to be 26.25 ± 5.40, 25.46 ± 6.0 and 25.54 ± 4.69 in the confirmed PROM, suspected PROM and the healthy control group respectively [10]. Ghasemi M *et al.* observed that average age of the study participants was 25.05±6 years in the confirmed PROM group and 25.85±5 years in the control group, with no statistically significant difference between the two groups [11].

In our study, most of the women were primigravida in both groups i.e, 60% in case group and 52.5% in control group. The mean values of gravida was 1.68±0.9 of the case group, and mean value of gravida of control was 2.03±1.1. Parity was 0.5±0.7 in cases and 0.7±0.8 in controls respectively. The P value was found to be not significant in both parameters between groups. These findings are in concordance with the study done by Kedar K *et al.* who observed mean gravida status of the case group and the control group was 1.91 ± 0.83 and 2.04 ± 0.83 respectively and the p-value was observed to be > 0.05 [12]. In our study, the mean gestational age in the case group was 38.43±1.2 weeks and in the control group was 39.0±1.4 weeks as per calculations from the dates of last menstrual period. As a result, no statistically significant difference between the groups was observed.

In our study the cases of low socioeconomic status were 80 % and middle socioeconomic status were 20%, and in control group 72.5 % in low socioeconomic status and 27.5 % in middle socioeconomic group, both groups were statistically not significant. Our study is comparable with the study by Shehla *et al.* which is 68.23% and 31.77 % respectively [13]. Studies shown that risk of PROM increases with decrease antibacterial activity in the amniotic fluid of patients with low socio-economic status due to associated factors like malnutrition, over exertion, poor hygiene, stress, high parity, recurrent UTI and anaemia. In our study, 50% of deliveries were booked and another 50% were unbooked in case group whereas 45 % deliveries were booked and 55 % were unbooked deliveries in control group. In our study, the only temperature parameter is significantly higher in case group than control (p<0.001) rest parameters were not significant. The mean value of temperature was 98.6±1.1 F in case group and 97.7 ± 0.6 F in control group. Similarly, Shruti Gupta *et al.* showed maternal fever can be one of the clinical features present in patients with PROM [14].

In our study, TLC (cumm) count among the cases

was12014.0±2782.1 as against which TLC (cumm) count was 9399.90±2148.2 in the control group The mean difference of TLC was found significant difference in both groups (p<0.05). TLC accuracy was 77.4%, with a cut-off value >9230/cumm, with sensitivity and specificity was 77.5% and 52.5% respectively. W Sereepapong *et al.* showed that among women with or without chorioamnionitis with PROM, TLC count in case was 15,000/cumm, sensitivity and specificity were 60 & 63 % respectively [15].

In our study, the mean level of amniotic fluid index (AFI) was highly significant lower in the case group than in the control group (5.3±1.7 vs. 11.3±1.5, p < 0.001) and the Vaginal fluid creatinine was highly considerably more in study group than control group (0.4±0.1 vs. 0.18±0.08, p < 0.001). The best cut-off concentrations for vaginal fluid creatinine and AFI were determined using receiver operating characteristic (ROC) curve analysis. The sensitivity & the specificity of vaginal fluid creatinine level to diagnose PROM (case) were 90% & 87.5% respectively. While it's overall accuracy was 91.6%, with a cut-off level > 0.25 mg/dl. The sensitivity & the specificity of amniotic fluid index (AFI) to diagnose PROM (case) were 97.5% & 92.5% respectively, while it's over all accuracy, was 99.3% respectively, with a cut-off level of ≤ 7.5 cm. Present study results are comparable with study performed by El-Garhy IT *et al.* who found the mean value of vaginal fluid creatinine concentrations in confirmed PROM and control groups using unpaired t test were0.70 ± 0.88 mIU/ml and 0.04 ± 0.18 mIU/ml, respectively, and the difference was highly statistically significant (p value < 0.001) and the mean AFI was 4.30 ± 1.64 cm in group PROM and 11.60 ± 2.60 cm in control group respectively, with highly significant difference between the two groups as regard to AFI (P value < 0.001). Creatinine level sensitivity and specificity were 72% and 94% respectively with a cut off value of 0.25 mg/ dl [16].

This was in accordance with Gada MS *et al.* showed that there was a considerable difference of creatinine level in the studied groups. The creatinine concentration was 0.64 ± 0.018 and 0.14 ± 0.006 mg/dl, in confirmed cases of PROM and controls, respectively. It was also observed that there was highly significant difference regarding AFI between the studied groups with the mean AFI of 5.30 ± 1.54 cm in group PROM and 11.06 ± 1.86 cm in control group respectively [17].

Conclusion

In our study the vaginal fluid creatinine was significantly higher in study group in comparison to control group. The cut off value of >0.25 mg/dl was observed to establish a diagnosis of PROM. The specificity, sensitivity & accuracy of vaginal fluid creatinine was 87.5 %, 90 % & 91.6 % respectively. The mean value of amniotic fluid index (AFI) was significantly lower in the case group than in the control group (5.3±1.7 vs. 11.3±1.5, p < 0.001). AFI was found to have highly significant association between the groups. (<0.001). The sensitivity & the specificity of amniotic fluid index (AFI) to diagnose PROM (case) were 97.5% & 92.5% respectively, while it's over all accuracy was 99.3% respectively, with a cut-off value of ≤ 7.5 cm. So to conclude that if on clinical examination diagnosis of PROM is doubtful or suspicious, USG for AFI can be first screening investigation that can be done to confirm leaking per vaginum, however there are other causes of reduced AFI or oligohydramnios such as placental insufficiency etc, so by adding vaginal fluid creatinine which is one of the diagnostic test for PROM we can increase the sensitivity and specificity to diagnose PROM. Vaginal fluid creatinine is a rapid, simple, inexpensive, non-invasive and widely available test, so that it can be easily incorporated in routine clinical use when the diagnostic dilemma of PROM is present. Moreover it can be used at primary health care settings. The lesser time taken to establish an accurate diagnosis would ensure prompt treatment and favourable maternal and fetal outcome. In patients with insignificant leaking and decreased AFI and with suspicion of PROM, vaginal fluid creatinine estimation may be useful for definitive diagnosis & institute appropriate management. Our study thereby indicates that estimating vaginal fluid creatinine levels may be useful to make an accurate diagnosis of PROM even at rural health care facilities and ensure early referral to tertiary care centres if needed.

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